A Highly Sensitive Electrochemical Determination of Isoniazid in Pharmaceutical Preparation by Differential Pulse Polarography

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Abstract

A simple strategy and highly sensitive electrochemical method was developed and described in Britton – Robinson buffer for isoniazid determination by Differential Pulse Polarography (DPP). A dropping mercury electrode (DME) was used to characterize the performance of the sensor. The reduction process on the (DME) gave a rise with one peak over E_p-1.17 v (vs. Ag/AgCl/), within the studied pH range (2 – 9), 3M KNO_3 as a supporting electrolyte and 4mm^3 mercury drop size at 25°C. A standard calibration plot was conducted in the range between 0.05- 0.40µg.ml^{-1}. The monitored differential pulse current was directly proportional to the concentration of Isoniazid and it showed a linear response in the studied range (correlation coefficient (r) = 0.9997) and the detection limit was 0.05µg. ml^{-1}. This method effectively applied in commercial Isoniazid pharmaceuticals.

Keywords: Isoniazid, pharmaceutical preparation, Differential pulse polarography.

I. INTRODUCTION

Isoniazid, also known as isonicotinylhydrazide (INH) (Fig. 1), is an antibiotic used as a first-line agent for the prevention and treatment of both latent and active tuberculosis.[1] It is effective against mycobacteria, particularly Mycobacterium tuberculosis. It is also active against some atypical types of mycobacteria, such as M. kansasii and M. xenopi.[2] Isoniazid is an organic compound that is available in tablet, syrup, and injectable forms.[3][4][5]. Isoniazid was first made in 1952. [6] [7] Three pharmaceutical companies unsuccessfully attempted to patent the drug at the same time, [8] the most prominent among them was Roche, which launched its version, Rimifon, in 1952.[9] With the introduction of isoniazid, a cure for tuberculosis was first considered possible. It is available worldwide, inexpensive, and is generally well tolerated. Isoniazid is on the World Health Organization's List of Essential Medicines, a list of medicines that constitute the bare minimum for a basic health system.[10][11][12]

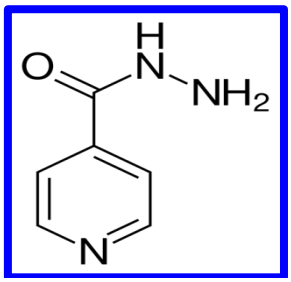


Fig. 1. Structure of Isoniazid

Polarography is the study of the electrolysis of solutions of electrooxidizable and or electro reducible substances between a dropping mercury electrode (DME) and some reference electrode (RE)[13][14]. The potential between these electrodes is varied and the consequent changes in the flow of current are measured. On plotting the changes in current flow versus the potential variation, one obtains an i - E curve known as polarograms [15][16]. The first scientist who discovered the use of the DME in electrolysis is Jaroslave Heyrovsky in 1922 who then received the Nobel Prize in Chemistry in 1959[17][18].

In differential pulse polarography, fixed magnitude pulses superimposed on a linear potential ramp - are applied to the working electrode at a time just before the end of the drop [19]. The current is measured twice at each mercury drop, before each pulse and at the end of the pulse time. The difference between the measurements Δi is plotted against the direct potential and produced peak - shaped polarograms, as Δi is largest for potential alterations in the region of the half - wave potential[20][21]. The formation of this difference also leads to a further reduction of the capacitive current contribution and therefore to an increase in sensitivity, even when compared with determinations by normal pulse polarography. The detection limit for determinations by differential pulse polarography is similar to that for square wave polarography at about (10^{-7} – 10^{-8} M) [22][23].

Many analytical methods have been developed for the determination of isoniazid,

including titrimetry [24], spectrophotometry [25][26], high performance liquid chromatography (HPLC) [27], chemiluminescence [28], fluorimetry [29], capillary electrophoresis [30] and electroanalytical methods [31]. Among these approaches, electroanalytical techniques are of particular advantage because of their practicality, simplicity, low-cost, good sensitivity, precision and rapidity for real-time detection.

II. EXPERIMENTAL

A. *Solutions and Reagents*

Isoniazid (analytical purity, Samarra drugs factory) was used without further purification. A stock solution of isoniazid was prepared with deionized water and kept in a refrigerator at about 4 ◦C. Isoniazid tablets (100 mg/tablet) were taken from Markets. Aqueous solutions were prepared with deionized water and stored in the shade. The 0.1M Britton – Robinson buffer(BR), within the pH range studied (2 – 9), and 3M KNO_3 as a supporting electrolyte also 4mm^3 mercury drop size at room temperature**.** A standard stock solution (100 µg. ml^{-1}) of isoniazid was prepared by dissolving an accurate mass of bulk drug in an appropriate volume of de-ionized water. More dilute solutions (0.05-/0.40 µg. ml^{-1}) were prepared daily by accurate dilution just before use. Isoniazid solutions were stable and their concentrations did not change with time. Britton-/Robinson (B-/R). Buffers of pH 2-9 [32] were prepared in deionized water. All the chemicals used were of analytical-reagent grade and were used without further purification.

B. *Apparatus*

All the electrochemical experiments were conducted in a three-electrode single compartment glass cell, including a DME electrode as working electrode, a platinum plate as auxiliary electrode, and an Ag/AgCl (3.0 mol L^{-1}KCl) as reference electrode. The polarographic measurements were carried out using a797 AV computrace (Metrohm, Herisau, Switzerland)**.**The sample solutions were purged with high-purity nitrogen gas for at least 5 min at the beginning of the experiments to remove oxygen. All measurements were carried out at room temperature.

C. *Preparation of Tablet Sample*

Ten tablets, each containing 100mg of isoniazid, were finely powdered respectively. The white powder was accurately weighed to 0.01 g and dissolved into 10mL deionized water. The mixture was shaken for 30 min and filtered into a 100mL volumetric flask. The residue was several times washed with deionized water and the solution was diluted to the mark.

III. RESULTS AND DISCUSSION

A. *Differential Pulse Polarography*

The DPP-polarograms for 0.4 µg. ml^{-1} isoniazid in B-/R. buffers of pH 2-/9 exhibited one irreversible cathodic peak. In B-/R. buffers of pH7, the height of the peaks shown in (Fig. 2).

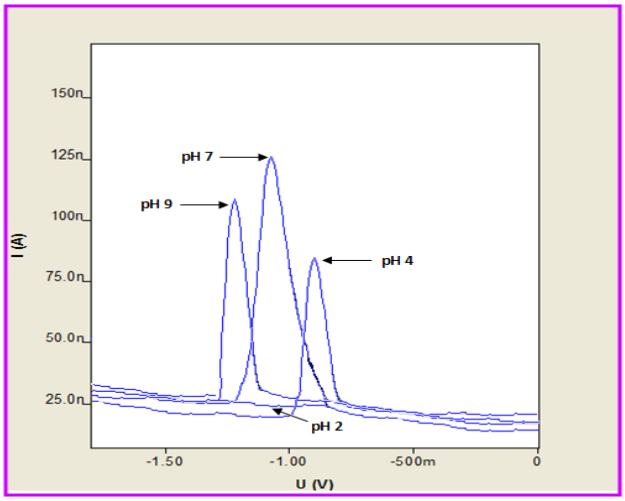


Fig. 2. DPP-Polarograms for 0.4 µg. ml^{-1} Isoniazid in B.R. Buffers of Different pH Values: (1) 2, (2) 4, (3) 7, (4) 9.

The peaks of isoniazid solution at different pH 2, 4, 7 and 9 showed a great effect on the diffusion current and half wave voltage, (Fig. 3). A weak cathode peak has been reached in the acid levels.

It has been found that pH 7 is the best level for isoniazid analysis. The reduction peak voltage resulting from pH showed that an electrochemical reaction exists consuming the hydrogen ions. A relationship between pH value and $E_{1/2}$ has been noticed, as values turn to be more negative when acidity increases. This proves that isoniazid electrochemical behavior depends on the pH of its solution.

These results suggested that the two-electron irreversible cathodic wave pH 7 may be attributed to the reduction of the C=O of the amide group.

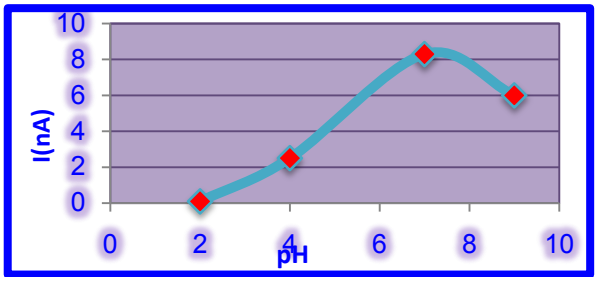


Fig. 3. pH Effect on the Value of Diffusion Current for Isoniazid.

B. Effect of Buffer Solutions

The effects of buffer solutions have been studied by choosing Britton–Robinson buffer, phosphate buffer and carbonate buffers solution at different pH levels. The results of the rise of the electric current value and the peak shape have been interpreted that Britton–Robinson buffer solution gives the best peak in comparison to the other buffer solutions when changing isoniazid buffer solutions, (Fig. 4). Moreover, results showed that B-R buffer solution at pH 7 gives the highest peak waves in comparison to other pH levels; therefore, it has been selected for the analysis, (Fig. 5).

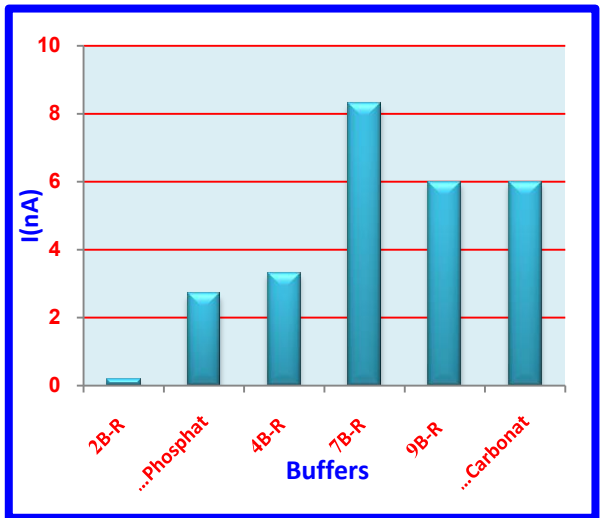
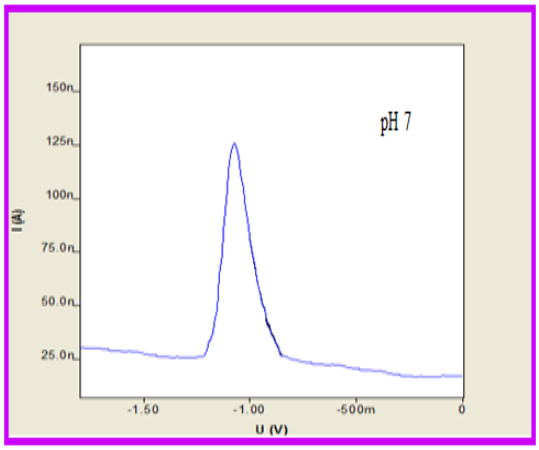


Fig. 4. Effect of Buffers Solution on the Reduction Peak of Isoniazid.



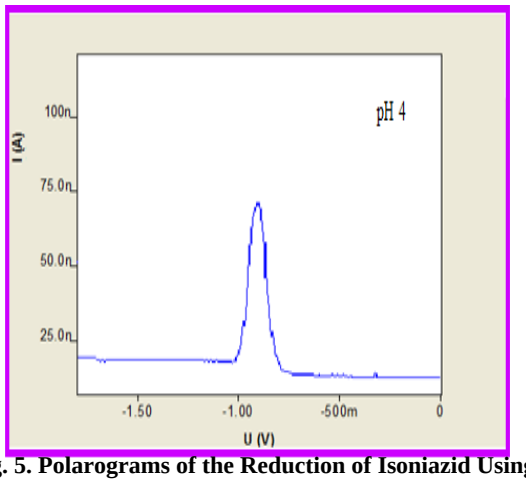


Fig. 5. Polarograms of the Reduction of Isoniazid Using Briton-Robinson (B-R) Buffers Solution, In Variable Ph Values 4, 7 And 9.

A 1–10.5 ml volume of the isoniazid solutions were analysis using Polarography device 797VA Computrace- Metrohm under the optimal experimental conditions found during this work. A standard calibration graph for isoniazid (Fig. 7) in the concentration range 0.05 to 0.40µg. ml^{-1} were prepared using the Method of Least Squares, M.L.S [33], and used to determine the amounts of isoniazid. The Proposed reduction mechanism of isoniazid showed in (Fig. 8).

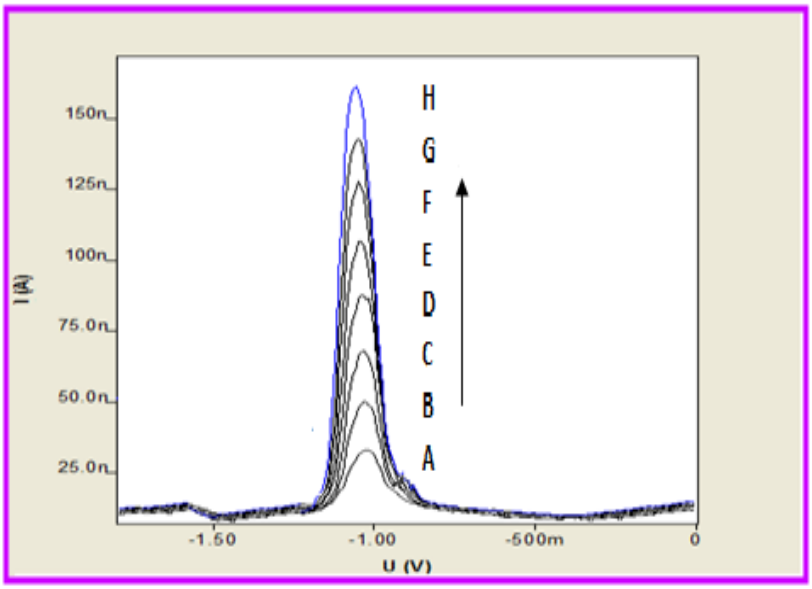


Fig .6. Polarogram of Different Solution of Isoniazid Drug by Using Britton–Robinson Buffer pH 7.

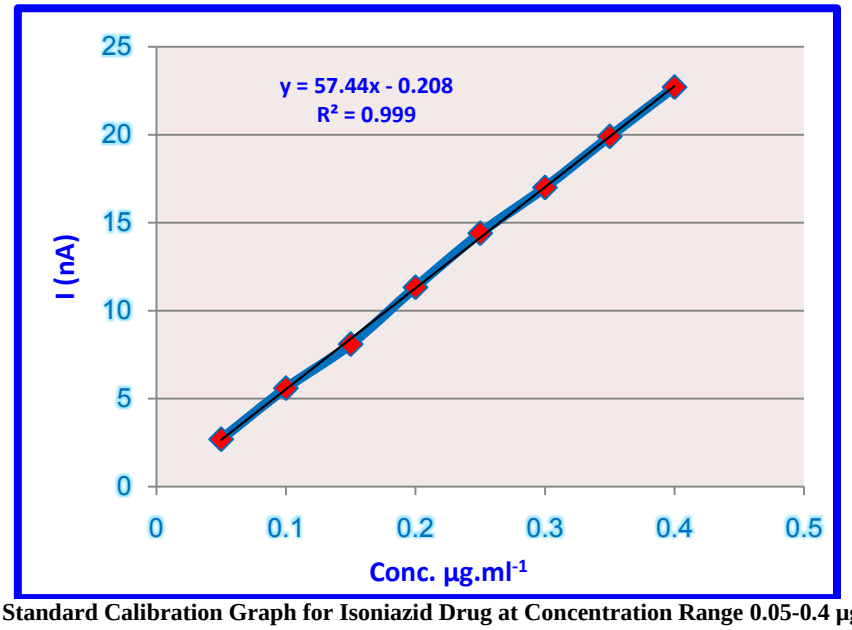


Fig. 7. Standard Calibration Graph for Isoniazid Drug at Concentration Range 0.05-0.4 µg. ml^{-1}.

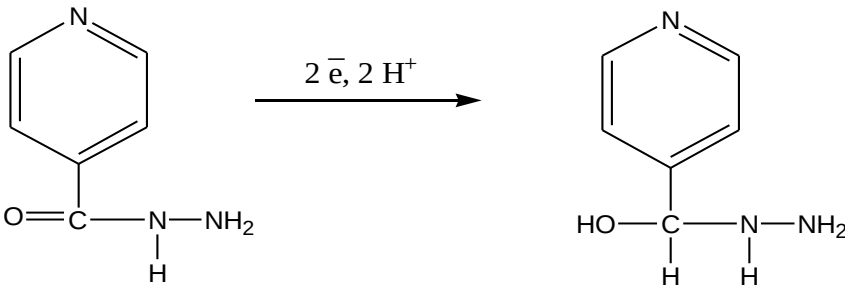


Fig .8. Proposed Reduction Mechanism of Isoniazid.

Table. 1. Optimum Analytical Conditions for Isoniazid Analysis.

Experimental condition	Variable	Optimum conditions
Solvent	Water, Ethanol, Methanol, Acetonitrile	Water
pH	2,4,7,9	7
Buffer Solution	Britton – Robinson(B-R) buffer Phosphate buffer Carbonate buffer	(B-R) buffer
Supporting Electrolyte	KNO_3, KCl, LiCl	KNO_3
Drop Size mm	2, 3, 4	4
Temperature (°C)	20, 25, 30, 35, 40, 45, 50	25

Table. 2. Analysis of Synthetic Made Isoniazid Sample.

Primary Conc. µg. ml^{-1}	Measured Current (nA)	Measured Conc. µg. ml^{-1}	Rec.%	AE	RE%	SD	RSD %
	22.70	0.3988	99.70	-0.0012	0.30		
	22.68	0.3984	99.60	-0.0016	0.40		
0.4	22.74	0.3995	99.87	-0.0005	0.12	0.001	0.250
	22.76	0.3998	99.95	-0.0002	0.05		
	22.61	0.3972	99.30	-0.0028	0.70		
	22.79	0.4003	100.07	0.0003	0.07		
	22.81	0.4007	100.17	0.0007	0.17		
	22.77	0.4000	100.00	0.0000	0.00		
	AV=22.73	AV=0.3993	AV=99.83				

Table. 3. Analysis of Commercial Pharmaceuticals Isoniazid.

Primary Conc. µg. ml^{-1}	Measured Current (nA)	Measured Conc. µg. ml^{-1}	Measurement of Drug	Rec.%	SD	RSD%
	22.77	0.4000	100	100		
	22.72	0.3991	99.77	99.77		
	22.68	0.3984	99.60	99.60		
0.4	22.77	0.4000	100	100	0.0008	0.20
	22.81	0.4007	100.17	100.17		
	22.70	0.3988	99.70	99.70		
	22.69	0.3986	99.65	99.65		
	22.71	0.3989	99.72	99.72		
	AV=22.73	AV=0.3993				

C. Limit of Detection and Limit of Quantification

The Limit of detection (LOD) and limit of quantification (LOQ) for the isoniazid was calculated using signal to noise ratio (S/N) of 3.3 and 10 respectively. The results were equal to 0.05 and 0.12 $\mu g. ml^{-1}$,respectively.

D. Accuracy and Precision

The accuracy and precision of the method for the determination of isoniazidwas tested. A 0.4 $\mu g.ml^{-1}$ synthetic isoniazidsample was prepared and analysis, the results shows absolute errors between -0.0016 to 0.0007 and relative errors ranging from -0.00 to 0.70 % with 0.001 SD and %RSD not exceed ± 0.250, Table.2.

IV. CONCLUSION

In the practical applications of this method several advantages were found, first; using DPP which gives sensitive and selective determination of isoniazid, and second, the developed methods proved to be rapid since the analysis of each sample required few minutes.

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REFERENCES

[1] Riccardi, Giovanna, and Maria RosaliaPasca. "Trends in discovery of new drugs for tuberculosis therapy." The Journal of antibiotics 67, no. 9 (2014): 655-659.

[2] Lougheed, Kathryn EA, Debra L. Taylor, Simon A. Osborne, Justin S. Bryans, and Roger S. Buxton. "New anti-tuberculosis agents amongst known drugs." Tuberculosis 89, no. 5 (2009): 364-370.

[3] Nguta, Joseph Mwanzia, Regina Appiah-Opong, Alexander K. Nyarko, Dorothy Yeboah-Manu, and Phyllis GA Addo. "Current perspectives in drug discovery against tuberculosis from natural products." International Journal of Mycobacteriology 4, no. 3 (2015): 165-183.

[4] Souayed, Nouha, MalekChennoufi, FidaBoughattas, Mohsen Hassine, Mosaddok Ben Attia, KarimAouam, Alain Reinberg, and Naceur A. Boughattas. "Circadian-time dependent tolerance and haematological toxicity to isoniazid in murine." Biomedicine & Pharmacotherapy 71 (2015): 233-239.

[5] Kumar, Piyush, PinkiRawat, and Pooja Singh. "Medicinal Chemistry And Development Of Antitubercular Drugs." International Journal of Pharmaceutical Sciences and Research 4, no. 6 (2013): 2080.

[6] Sharma, Moni, and PoojaSapra Sharma. "Chemistry & Biology Interface." Chemistry & Biology 3, no. 2 (2013): 116-122.

[7] Shen, Shujie. "The Antimycobacterial Activity of Hypericumperforatum Herb and the Effects of Surfactants." (2012).

[8] Miller, Christopher Hamilton. "Seleno-amino acids: A novel class of anti-tuberculosis compounds identified through modified culture screening conditions." (2013).

[9] Moody, David Branch, Emilie Layre, and David C. Young. "Methods and systems for determining m. tuberculosis infection." U.S. Patent Application 14/900,725, filed June 26, 2014.

[10] Timmins, Graham S., and VojoDeretic. "Mechanisms of action of isoniazid." Molecular microbiology 62, no. 5 (2006): 1220-1227.

[11] Kumar, Shashank, Ramesh Kumar, AsthaDwivedi, and Abhay K. Pandey. "In vitro antioxidant, antibacterial, and cytotoxic activity and in vivo effect of Syngoniumpodophyllum and Eichhorniacrassipes leaf extracts on isoniazid induced oxidative stress and hepatic markers." BioMed research international 2014 (2014).

[12] Mansuri, Shakeel S., AbhishekPathak, and Sadhana J. Rajput. "Development and validation of chemometric assisted UV Spectrophotometric and RPLC-PDA methods for the simultaneous In Vitro analysis of Isoniazid, Rifampicin and Piperine In Their Pharmaceutical Formulation." IAJPR 4, no. 1 (2014): 540-553.

[13] Zuman, Petr. Topics in organic polarography. Springer Science & Business Media, 2012.

[14] Yilmaz, Ümmihan T., and ZehraYazar. "Determination of melamine by differential pulse polarography/application to milk and milk powder." Food Analytical Methods 5, no. 1 (2012): 119-125.

[15] Escarpa, Alberto. "Food electroanalysis: sense and simplicity." The Chemical Record 12, no. 1 (2012): 72-91.

[16] Asadpour-Zeynali, Karim, and PayamSoheili-Azad. "Simultaneous polarographic determination of 2-nitrophenol and 4-nitrophenol by differential pulse polarography method and support vector regression." Environmental monitoring and assessment 184, no. 2 (2012): 1089-1096.

[17] Lubert, Karl-Heinz, and Kurt Kalcher. "History of electroanalytical methods." Electroanalysis 22, no. 17-18 (2010): 1937-1946.

[18] Zuman, Petr. "Electrolysis with a dropping mercury electrode: J. Heyrovsky's contribution to electrochemistry." Critical reviews in analytical chemistry 31, no. 4 (2001): 281-289.

[19] Heyrovský, Michael. "Ninety years of polarography." The Chemical Record 12, no. 1 (2012): 14-16.

[20] Kocak, S., O. Tokusoglu, and S. Aycan. "Some heavy metal and trace essential element detection in canned vegetable foodstuffs by differential pulse polarography (DPP)." Electronic J. Environ. Agric. Food Chem 4 (2005): 871-878.

[21] Kissinger, Peter, and William R. Heineman, eds. Laboratory Techniques in Electroanalytical Chemistry, revised and expanded. CRC press, 1996.

[22] Ghoneim, M. M., A. M. Beltagi, and A. Radi. "Square-wave adsorptive stripping voltammetric determination of the anti-inflammatory drug lornoxicam." Analytical sciences 18, no. 2 (2002): 183-186.

[23] Yun Xia, Hao, and Xiao Ya Hu. "Determination of isoniazid using a gold electrode by differential pulse voltammetry." Analytical letters 38, no. 9 (2005): 1405-1414.

[24] El-Brashy, A. M., and S. M. El-Ashry. "Colorimetric and titrimetric assay of isoniazid." Journal of pharmaceutical and biomedical analysis 10, no. 6 (1992): 421-426.

[25] Goicoechea, Héctor C., and Alejandro C. Olivieri. "Simultaneous determination of rifampicin, isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration." Journal of pharmaceutical and biomedical analysis 20, no. 4 (1999): 681-686.

[26] Nagaraja, P., KC Srinivasa Murthy, and H. S. Yathirajan. "Spectrophotometric determination of isoniazid with sodium 1, 2-naphthoquinone-4-sulphonate and cetyltrimethyl ammonium bromide." Talanta 43, no. 7 (1996): 1075-1080.

[27] Khuhawar, M. Y., and F. M. A. Rind. "Liquid chromatographic determination of isoniazid, pyrazinamide and rifampicin from pharmaceutical preparations and blood." Journal of Chromatography B 766, no. 2 (2002): 357-363.

[28] Safavi, Afsaneh, Mohammad Ali Karimi, and Mohammad Reza HormoziNezhad. "Flow injection determination of isoniazid using N-bromosuccinimide-and N-chlorosuccinimide-luminolchemiluminescence systems." Journal of pharmaceutical and biomedical analysis 30, no. 5 (2003): 1499-1506.

[29] Lapa, Rui AS, José LFC Lima, and João LM Santos. "Fluorimetric determination of isoniazid by oxidation with cerium (IV) in a multicommutated flow system." Analyticachimicaacta 419, no. 1 (2000): 17-23.

[30] Liu, Ying, Zhifeng Fu, and Lin Wang. "Capillary electrophoresis analysis of isoniazid using luminol-periodate

potassium chemiluminescence system." Luminescence 26, no. 6 (2011): 397-402.

[31] Majidi, Mir Reza, AbolghasemJouyban, and KarimAsadpour-Zeynali. "Voltammetric behavior and determination of isoniazid in pharmaceuticals by using overoxidizedpolypyrrole glassy carbon modified electrode." Journal of Electroanalytical Chemistry 589, no. 1 (2006): 32-37.

[32] Lastra, Olga C., Igor G. Lemus, Hugo J. Sánchez, and Renato F. Pérez. "Development and validation of an UV derivative spectrophotometric determination of Losartan potassium in tablets." Journal of pharmaceutical and biomedical analysis 33, no. 2 (2003): 175-180.

[33] Lawson, Charles L., and Richard J. Hanson. Solving least squares problems. Vol. 15. Philadelphia: Siam, 1995.